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(71) Applicant *(for all designated States except US)*: ZENECA LIMITED [GB/GB]; 15 Stanhope Gate, London W1Y 6LN (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: MAY, Gregory, Dewitt [US/US]; Boyce Thompson Institute for Plant Research Inc., Tower Road, Ithaca, NY 14853 (US). KIPP, Peter, Barber [US/US]; Boyce Thompson Institute for Plant Research Inc., Tower Road, Ithaca, NY 14853 (US).

(74) Agents: HUSKISSON, Frank, Mackie et al.; Zeneca Agrochemicals, Intellectual Property Dept., Jealott's Hill Research Station, P.O. Box 3538, Bracknell, Berkshire RG42 6YA (GB).

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(54) Title: GENE PROMOTER SEQUENCE FROM BANANA

(57) Abstract

The promoter of the 1-aminocyclopropane-1-carboxylic acid oxidase gene in banana has the nucleotide sequence SEQ-ID-NO-1 and is used for driving expression of foreign genes in transgenic plants.

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GENE PROMOTER SEQUENCE FROM BANANA

The present invention relates to a gene promoter sequence isolated from banana and to the use of that promoter to regulate expression of chimeric genes in plants.

Gene expression is controlled by various regulatory components, including nucleic acid and protein elements. In particular, gene expression is controlled by a region commonly referred to as the "promoter" which lies upstream (5') of the protein encoding region. A promoter may be constitutive or tissue-specific, developmentally-regulated and/or inducible.

Within the promoter region there are several domains which are necessary for full function of the promoter. The first of these domains lies immediately upstream of the structural gene and forms the "core promoter region" containing consensus sequences, normally 70 base pairs immediately upstream of the gene. The core promoter region contains the characteristic CAAT and TATA boxes plus surrounding sequences, and represents a transcription initiation sequence which defines the transcription start point for the structural gene. The precise length of the core promoter region is indefinite but it is usually well-recognisable. Such a region is normally present, with some variation, in all promoters. The base sequences lying between the various well-characterised "boxes" appear to be of lesser importance.

The presence of the core promoter region defines a sequence as being a promoter: if the region is absent, the promoter is non-functional. Furthermore, the core promoter region is insufficient to provide full promoter activity. A series of regulatory sequences upstream of the core constitute the remainder of the promoter. The regulatory sequences determine expression level, the spatial and temporal pattern of expression and, for an important subset of promoters, expression under inductive conditions (regulation by external factors such as light, temperature, chemicals, hormones).

Manipulation of crop plants to alter and/or improve phenotypic characteristics (such as productivity or quality) requires the expression of heterologous genes in plant tissues. Such genetic manipulation therefore relies on the availability of means to drive and to control gene expression as required; for example, on the availability and use of suitable promoters which are effective in plants and which regulate gene expression so as to give the desired effect(s) in the transgenic plant. It is advantageous to have the choice of a variety of different promoters so that the most suitable promoter may be selected for a particular gene, construct, cell, tissue, plant or environment.

Promoters (and other regulatory components) from bacteria, viruses, fungi and plants have been used to control gene expression in plant cells. Numerous plant transformation experiments using DNA constructs comprising various promoter sequences fused to various foreign genes (for example, bacterial marker genes) have led to the identification of useful promoter sequences. It has been demonstrated that sequences up to 500-1000 bases in most instances are sufficient to allow for the regulated expression of foreign genes. However, it has also been shown that sequences much longer than 1 kb may have useful features which permit high levels of gene expression in transgenic plants. A range of naturally-occurring promoters are known to be operative in plants and have been used to drive the expression of heterologous (both foreign and endogenous) genes in plants: for example, the constitutive 35S cauliflower mosaic virus promoter, the ripening-enhanced tomato polygalacturonase promoter (Bird et al, 1988, Plant Molecular Biology, 11:651-662), the E8 promoter (Diekman & Fischer, 1988, EMBO, 7:3315-3320) and the fruit specific 2A11 promoter (Pear et al, 1989, Plant Molecular Biology, 13:639-651) and many others.

As stated above, successful genetic manipulation relies on the availability of means to control plant gene expression as required. The scientist uses a suitable expression cassette (incorporating one or more promoters and other components) to regulate gene expression in the desired manner (for example, by enhancing or reducing expression in certain tissues or at certain developmental stages). The

ability to choose a suitable promoter from a range of promoters having differing activity profiles is thus important.

In work leading to the present invention, we have isolated and fully sequenced an ACC oxidase gene promoter from banana. ACC oxidase is an enzyme involved in the biosynthesis of ethylene. In this document the abbreviation "ACC" means 1-aminocyclopropane-1-carboxylic acid.

Ethylene is a major plant hormone which has been shown to have a variety of effects on plant growth and development in many species. Endogenous levels of ethylene increase during several stages of development and in response to various stimuli including mechanical wounding and pathogen infection, ripening of climacteric fruits and leaf and flower senescence. The biosynthetic pathway for ethylene in plants is well-established; for example, a review of ethylene biosynthesis was published by Yang and Hoffman in 1984 (Annual Review Plant Physiology, 35:155-189). The final stages of ethylene biosynthesis proceed by the following pathway:

Methionine → S-adenosyl-L-methionine (SAM) → 1-aminocyclopropane-1-carboxylic acid (ACC) → Ethylene.

The final step in the pathway of ethylene biosynthesis is the conversion of the cyclic amino acid 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene. This reaction is catalysed by the enzyme ACC oxidase (also known as "ethylene forming enzyme" or EFE) which was once thought to be constitutively expressed in most tissues. However, since the cloning of the gene the messenger RNA has been shown to be induced under a number of conditions known to result in increased ethylene production.

After the cloning of the first ACC oxidase cDNA clone from tomato, standard hybridisation procedures were used to isolate clones for ACC oxidase from

other plant species. ACC oxidase cDNA or genomic clones have now been isolated from at least nine other species:

(1) Melon (*Cucumis melo*)
Balague et al, 1993, Eur J Biochem, 212:27-34;

(2) Petunia (*Petunia hybrida*)
Wang and Woodson, 1992, Plant Physiol, 100:535-536;

(3) Apple (*Malus domestica*)
Ross et al, 1992, Plant Molecular Biology, 19:231-238;

(4) Mustard (*Brassica juncea*)
Pua et al, 1992, Plant Molecular Biology, 19;541-544;

(5) Avocado (*Persea americana*)
Christofferson et al, 1993, Cellular and molecular aspects of the plant hormone ethylene, Pech JC et al (eds), Kluwer, pages 65-71;

(6) Peach (*Prunus persica*)
Callahan et al, 1992, Plant Physiol, 100:482-488;

(7) Orchid (*Phalaenopsis*)
Nadeau et al, 1993, Plant Physiol, 103:31-39;

(8) Kiwifruit (*Actinidia deliciosa*)
Macdiarmid and Gardiner, 1993, Plant Physiol, 101:691-692;

(9) Carnation (*Dianthus caryophyllus*)
Wang et al, 1991, Plant Physiol, 96:1000-1001.

The whole or part of the protein coding regions of ACC oxidase genes may be incorporated into DNA constructs for plant transformation. International patent application publication number WO91/01375 describes a method of modifying ethylene biosynthesis in plants by using DNA constructs based on genes encoding an enzyme involved in ethylene biosynthesis (such as ACC oxidase). Sense constructs as well as antisense constructs may be used to regulate gene/enzyme activity.

An object of the present invention is to provide alternative promoters capable of driving gene expression in plants.

According to the present invention, there is provided a DNA sequence encoding a banana ACC oxidase gene promoter capable of driving gene expression in plants having the sequence shown in SEQ ID NO 1 extending from nucleotide number 93 to 1448, or active variants thereof.

"Active variants" are DNA sequences partially homologous to SEQ ID NO1 or fragments thereof which retain promoter activity. It may be possible to alter the level or type of activity of the Banana ACC oxidase promoters by manipulating their sequences: for example, by altering the nucleotide sequence in key regulatory regions, by truncating the sequence or by deleting parts within the sequence. Segments of the Banana ACC oxidase promoter sequences of between 100 and 2000 bases in length may be useful as plant-operative promoters.

The promoters of the invention are suitable for incorporation into DNA constructs encoding any target gene so that the target gene is expressed when the construct is transformed into a plant.

The nucleotide sequence of the banana ACC oxidase promoter is shown as SEQ ID NO 1: the ATG start codon is shown at the end of the promoter sequence (base number 1449 to 1451). The putative TATA-box is between base number 1368 and base number 1373

The sequence of the banana ACC oxidase promoter has not previously been elucidated. Example 1 gives information on the limited homology between the banana ACC oxidase promoter and known promoters.

The banana ACC oxidase promoter may be synthesised *ab initio* using the sequence shown in SEQ ID NO 1 as a guide. Alternatively, the promoter may be isolated from plant genomic DNA libraries using suitable probes derived from the said sequences or the promoter may be isolated using a PCR approach.

In practice the promoter of the invention may be inserted as a promoter sequence in a recombinant gene construct destined for use in a plant. The construct is then inserted into the plant by transformation. Any plant species may be transformed with the construct, and any suitable transformation method may be employed.

According to a second aspect of the invention, there is provided a plant gene expression cassette comprising the banana ACC oxidase promoter operatively linked to a target gene, the promoter having the sequence shown as SEQ ID NO 1 or active variants thereof.

The target gene is a DNA sequence which may be derived from an endogenous plant gene or from a foreign gene of plant, fungal, algal, bacterial, viral or animal origin. Normally it is a sequence other than the sequence encoding the ACC oxidase protein which follows the Banana ACC oxidase promoter in the naturally-occurring Banana ACC oxidase gene. The target gene may be a single gene or a series of genes. The target gene is adapted to be transcribed into functional RNA under the action of plant cell enzymes such as RNA polymerase. Functional RNA is RNA which affects the biochemistry of the cell: for example, it may be mRNA which is translated into protein by ribosomes or it may be RNA which inhibits the translation of mRNA related to it. Thus the target gene sequence may be a sense sequence encoding at least part of a functional protein or an antisense sequence.

The expression cassette is suitable for general use in plants. In practice the DNA construct comprising the expression cassette of the invention is inserted into a plant by transformation. Any transformation method suitable for the target plant or plant cells may be employed, including infection by *Agrobacterium tumefaciens* containing recombinant Ti plasmids, electroporation, microinjection of cells and protoplasts, microprojectile transformation, pollen tube transformation and transformation of plant cells using mineral fibres (US Patent Number 5302523,

International Patent Application Publication Number WO94/28148). The transformed cells may then in suitable cases be regenerated into whole plants in which the new nuclear material is stably incorporated into the genome. Both transformed monocotyledonous and dicotyledonous plants may be obtained in this way. Transgenic plant technology is for example described in the following publications: Swain WF, 1991, TIBTECH 9: 107-109; Ma JKC et al, 1994, Eur J Immunology 24: 131-138; Hiatt A et al, 1992, FEBS Letters 307:71-75; Hein MB et al, 1991, Biotechnology Progress 7: 455-461; Duering K, 1990, Plant Molecular Biology 15: 281-294.

Examples of genetically modified plants which may be produced include but are not limited to field crops, cereals, fruit and vegetables such as: canola, sunflower, tobacco, sugarbeet, cotton, soya, maize, wheat, barley, rice, sorghum, mangoes, peaches, apples, pears, strawberries, bananas, melons, potatoes, carrot, lettuce, cabbage, onion.

The invention further provides a plant cell containing a gene expression cassette according to the invention. The gene expression cassette may be stably incorporated in the plant's genome by transformation. The invention also provides a plant tissue or a plant comprising such cells, and plants or seeds derived therefrom.

The invention further provides a method for controlling plant gene expression comprising transforming a plant cell with a plant gene expression cassette having an Banana ACC oxidase promoter operatively linked to a target gene, whereby the activated promoter drives expression of the target gene. The promoter may be activated under certain spatial, temporal, developmental and/or environmental conditions.

In order to determine their temporal and spatial expression, the promoter fragments of the Banana ACC oxidase genes are fused to the GUS (β-glucuronidase) reporter gene in DNA constructs suitable for plant transformation. GUS

accumulation in transgenic plants may then be monitored. Example 3 describes some of these experiments.

The invention will now be described by way of example.

EXAMPLE 1

Isolation and characterisation of banana ACC oxidase promoter

A banana genomic DNA library prepared in the vector Lambda Fix II (Stratagene, La Jolla, CA) was probed with the apple ACC oxidase cDNA clone Apy4 (Ross et. al. 1992, Plant Molecular Biology, 19: 231-238). Six clones containing sequences with homology to the apple ACC oxidase cDNA were identified by hybridisation. A 3365bp SalI fragment from one of the genomic clones - Banana ACC oxidase genomic clone 4 - was further characterised by sequence analysis.

Homology between clone 4 and the coding sequences of ACC oxidase genes from other species confirmed the identity of the genomic clone. Clone 4 contained the entire ACO coding sequence (interrupted by 3 introns) as well as the associated 5' and 3' proximal regions. The clone contained 1448 bases 5' of the predicted start codon (ATG) of the ACO coding sequence. This putative promoter sequence (SEQ ID No 1) contains several regions of dyad symmetry and direct repeats.

EXAMPLE 2

Analysis of expression patterns of banana ACC oxidase gene.

Fruit ripening enhanced expression of ACC oxidase genes was demonstrated in banana pulp and peel. Total RNA was isolated from fruit pulp and peel samples of green bananas and 6 developmental stages after ripening had been induced by treatment with ethylene (>100µl/l for 24 hr).

10µg of RNA from each ripening stage and tissue were separated on formaldehyde denaturing gels and blotted onto nylon membrane. The membranes were pre-hybridised in 5x Denhardts, 5X SSC for 2hr at 65° and then hybridised overnight with random prime labelled banana ACO cDNA ACOS7 (Patent application No.) at 65°C in fresh 5x Denhardts, 5X SSC. The membranes were then washed in 3x SSC, 0.1% SDS for 20 mins at 65°C and subseqently in 0.3x SSC, 0.1% SDS for 20 mins at 65°C prior to exposure to X-Ray film.

For both pulp and peel samples, there was very weak hybridisation to RNA from green unripe fruit. However, there was strong hybridisation of the ACO probe to pulp and peel RNA samples from all stages of ripening.

EXAMPLE 3

Banana ACC oxidase promoter - GUS constructs for analysis in transgenic plants

The 1386 bp HindIII fragment (93 to 1479)fragment from the banana ACC oxidase genomic clone 4 was ligated into the HindIII site of plant expression vector pBI101 (Clonetech, Palo Alto, CA, USA). This construction created a translational fusion between the banana ACC oxidase promoter and the *E. coli* β-glucuronidase (GUS) reporter gene:

```
1449-AGAGCGTGTC ATG GAT TCC TTT CCG GTT ATC GAC ATG GAG
                Met Asp Ser Phe Pro Gly Ile Asp Met Glu

HindIII-AAG CTT GCA TGC CTG CAG GTC GAC TCT AGA GGA TCC
        Lys Leu Ala Cys Leu Gln Val Asp Ser Arg Pro Ser

        CCG GGT GGT CAG TCC CTT ATG -
        Pro Gly Gly Gln Ser Leu Met - GUS
```

Transformed banana plants containing the ACC oxidase promoter / GUS construct are produced by the method described in May et al (Biotechnology, 13,

486- 492, 1995). Transgenic plants are regenerated and grown to maturity. Fruit ripening enhanced expression of GUS is demonstrated by assay of enzyme activity in tissues at various stages of maturity.

SEQ ID No: 1

SEQUENCE TYPE: Nucleotide

SEQUENCE LENGTH: 1484bp

STRANDEDNESS: Single

TOPOLOGY: Linear

MOLECULE TYPE: DNA

ORIGINAL SOURCE ORGANISM: Banana (*Musa acuminata*), Cavendish

IMMEDIATE EXPERIMENTAL SOURCE: Lambda ACC Oxidase clone 4

FEATURES:

From: 92 To: 97	HindIII site
From: 1449 To: 1451	Putative start codon (ATG)
From: 1479 To: 1484	HindIII site

```
CGGCAAGTCA TGCATAGCTG CAAACATGTG ACAGGCACCG AACCAACAAT TGAAGAAGAT   60
ACGATAAACA TGCGTGAGCC TACATGCACC AAAGCTTGCC GACAAGTCAT GTTTGGGTGC  120
ACAATGTGTC CTCATCTTAC TTGCATATCT GCTGTTGCAC AACAGCAGAT TGCATGGAGG  180
TGTGTTTTCC GGCAATGCAA TCTTTGATGT TGGTTCTCTT NNCTCTCTTC TTGGATTGTT  240
TATAGCTCTG TTTCTTGTGC TCTTCTTTNA CGTAGATTCA TAGCGTAGCT TAAGTTGTTA  300
TAGATTACCT GTTTTACTGG GCAAACTTGT GCAACCCAGG AATATTCCCA TGTGCATCTT  360
CTTCCTGTTT CCTCTNTCAA ACTGTCTGTC ATGATGAGGN AGNACCGCTC TNGAGAATTC  420
CNATGTGGNT GGTTTACCCA TAACTGAGAG ATNTGTTGGC GTTCANTACA TGATGNTGTG  480
TCAGAANCAA TCTACCTGTT CTGGCCACGG AGGGTTTATG CGCAGTTCAA CGCCATTGGT  540
GGTGGTGTGG CTGCGTAATC CTTGGCTCCG TGCCCACGCA CATGACGAAC CCATTGTTTT  600
TTTGTGGCCA CCAACCGGAG AAGGGAGTCA AATAACTAGC GGACGGGAAT TTTCCCTTGA  660
CTTGTTCACT CACGTAAAGT GGTGAATTGG TAAAATTAAA CGGATCTATG GTCGGAGGAT  720
TAAGAAAAAC CCAGATAAGG GAGACCCATC CTTCACAAGT TGGACCTCGG CCGATTNGGC  780
CCGATCACCC TNTTTCACAC CGGATACTTA GTTTACGGCC ATTGCCGAAT GCCGACAACC  840
ATCGAGCGTT GTATTTAAGC AGGATGGCCC ATTTCTCAAA AACGAGAGGG ATACGAGTGG  900
AAAGGCCCTC TAATGAGCTG TGAACCGAAA CAATTTCTAC CCTATCGATC CCTGTTCTTT  960
TGATATGAAG TATACCCAAC AGTTCAAGAG AAGACGAGTA CACACGCATC GCCGATGCTG 1020
TGACGTTACT TTCTGAGTTG GCAATTTTGT CACTACAATC CAAGCGGAAG CCATGCACGC 1080
GAGGCGTCGC CATGGAAGAA CTCAACAACA TGATGCCTTC CCGGGTCTCC TCAAAGGGGA 1140
GAGACCGATG GAAGCAGCCA AACTTGGTCC CCGATCGTGA TGGGACGCGA GAGGTGGAAG 1200
CAAGGAGGGT GGAGAACCAG GCCAAAGGTG GTGGGGCTGA GAGATGGCCA ACTGGGTCAC 1260
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CTTATGGAAT CGGCTCCGTT ACGTCTTCCA CTGCTGTTGC TCTCGTCGAT AGATCCTTCT 1320
CCAACTTTGC TTCTTCACTC ATTTCGTCCC TCGACGTCAA GAACGCCTAT AAATTGCCTG 1380
GTAATCAGCA GCACCTAGCA CACTCCAGAT AGAAAGCACA AGTGCAATCA GGGAAGAAAG 1440
AGCGTGTCAT GGATTCCTTT CCGGTTATCG ACATGGAGAA GCTT 1484

CLAIMS

1. A DNA sequence encoding a banana ACC oxidase gene promoter capable of driving gene expression in plants having the sequence shown in SEQ ID NO 1 extending from nucleotide number 93 to 1448, or active variants thereof.

2. A DNA construct comprising a promoter as claimed in claim 1 operatively linked to a transcribable DNA region and a transcription termination signal.

3. A transgenic plant having stably incorporated within its genome a DNA construct as claimed in claim 2.

4. A plant gene expression cassette comprising, in sequence, the banana ACC oxidase promoter claimed in claim 1, the coding region of a target gene and a 3' polyadenylation signal.

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/GB 97/00654

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/29 C12N15/82 C12N9/02 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL DATABASE, 11 September 1995, HEIDELBERG, XP002033394 LOPEZ-GOMEZ R. ET AL.: "AC X91076" see the whole document ---	1-4
A	WO 91 01375 A (ICI PLC) 7 February 1991 see the whole document ---	1-4
A	PLANT MOLECULAR BIOLOGY, vol. 19, no. 2, May 1992, pages 231-238, XP002033393 ROSS G. ET AL.: "An ethylene-related cDNA from ripening apples" cited in the application see the whole document ---	1-4

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Inte. nal Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category '	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	BIOCHEMISTRY AND MOLECULAR BIOLOGY INTERNATIONAL, vol. 41, no. 5, 1997, pages 941-950, XP000675954 HUANG P. ET AL.: "Characterization and expression analysis of a banana gene encoding 1-aminocyclopropane-1-carboxylate oxidase" see the whole document ---	1-4
P,X	PLANT SCIENCE, vol. 123, no. 1-2, 1997, pages 123-131, XP000676021 LOPEZ-GOMEZ R. ET AL.: "Ethylene biosynthesis in banana fruit: Isolation of a genomic clone to ACC oxidase and expression studies" see the whole document -----	1-4

2

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/GB 97/00654

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9101375 A	07-02-91	AU 627063 B	13-08-92
		AU 6042390 A	22-02-91
		EP 0482053 A	29-04-92
		JP 4506602 T	19-11-92
		US 5530190 A	25-06-96
		US 5365015 A	15-11-94

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

idem as subject 1, by using nucleotide sequences homologous to a Histone H1, namely SEQID No. 68.

19. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences homologous to a Wali 7 Protein, namely SEQID No. 69.

20. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences with unknown function, namely SEQID No. 53.

21. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences with unknown function, namely SeqID 54.

22. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences with unknown function, namely SeqID 55.

23. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences with unknown function, namely SeqID 56.

24. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences with unknown function, namely SeqID 57.

25. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences with unknown function, namely SeqID 58.

26. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences with unknown function, namely SeqID 59.

27. Claims: 1-7 partially

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

idem as subject 1, by using nucleotide sequences homologous to Legumin storage proteins, namely SEQID No. 43-45.

10. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences homologous to Isoflavonoid Reductases, namely SEQID No. 46 and 47.

11. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences homologous to Extensins, namely SEQID No. 48.

12. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences homologous to Chitinases, namely SEQID No. 49.

13. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences homologous to PEP carboxylases, namely SEQID No. 50.

14. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences homologous to Beta 1,3-glucanase Regulator gene, namely SEQID No. 51.

15. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences homologous to a High Mobility Group Protein, namely SEQID No. 52.

16. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences homologous to an Elongation factor EF-1-alpha, namely SEQID No. 64 and 66.

17. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences homologous to a Heat Shock Protein, namely SEQID No. 67.

18. Claims: 1-7 partially

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

idem as subject 1, by using nucleotide sequences with unknown function, namely SeqID 60.

28. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences with unknown function, namely SeqID 61.

29. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences with unknown function, namely SeqID 62.

30. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences with unknown function, namely SeqID 63.

31. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences with unknown function, namely SeqID 65.

32. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences with unknown function, namely SeqID 70.

33. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences with unknown function, namely SeqID 71.

34. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences with unknown function, namely SeqID 72.

35. Claims: 1-7 partially

idem as subject 1, by using nucleotide sequences with unknown function, namely SeqID 73.

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